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Cattle as Biomonitors of Soil Arsenic, Copper, and Zinc Concentrations in Galicia (NW Spain)

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Abstract. Determination of soil concentrations of trace and pollutant metals over large spatial areas requires laborious and expensive sampling effort. In this study, we examined the feasibility of using calves as biomonitors of soil semimetal and trace metal concentrations in Galicia (NW Spain), a region in which calves are predominantly reared on grass or locally grown forage. We determined the concentrations of arsenic, copper, and zinc in the liver, kidney, muscle, and blood of calves from across Galicia and related them to the metal concentrations in the soil from the areas in which the animals were reared. For each element, liver (but not usually kidney, muscle, or blood) concentrations were significantly elevated in animals from areas with higher soil concentrations. Liver arsenic concentrations were only markedly greater in animals from areas with soil arsenic levels ff 20 mg/kg, and calves may not be sensitive enough biomonitors of background variation in soil levels, although they may be useful for monitoring anthropogenic arsenic contamination. Copper and zinc liver levels increased progressively with soil levels, and the pattern was especially marked for copper. The relatively unusual copper metabolism of cattle and other ruminants may make them particularly good biomonitors for environmental concentrations of this metal.

Various animal species have been suggested as suitable biomonitors of trace and pollutant metal levels in the terrestrial environment (MARC 1990; O'Brien *et al.* 1993). The use of such biomonitors has the advantage over analysis of soil, air, and water in that it provides a measure of exposure that is integrated spatially, temporally, and across media (Talmage and Walton 1991). Where human health is the primary concern, biomonitors should ideally be (1) widely distributed to allow cross-habitat comparisons but territorial and nonmigratory so that measured concentrations can be linked to source areas; (2) numerous and relatively large so that it is feasible to collect sufficient numbers of samples for chemical and other (such as histological) analysis; (3) near the top of a food chain so that any biomagnification is detected; (4) relatively long-lived and broadly similar to humans in their physiological and toxicological responses to contaminants if they are to be used to predict the likely effects of short-and long-term exposure (Holden 1973; Landres *et al.* 1988; O'Brien *et al.* 1993, Esselink *et al.* 1995). It has been argued that animals in livestock production largely fulfill these criteria (Ronneau *et al.* 1983; Ronneau and Cara 1984; Morcombe *et al.* 1994; Petersson Grawé *et al.* 1997), and, in rural areas, this is likely to be true if livestock are reared on grass or locally grown fodder. In addition, domestic animals have the advantage that they are an important source of food for humans and so provide a direct measure of pollutant transfer to humans (O'Brien *et al.* 1993).

In Galicia (NW Spain), cattle are the most numerous of the different kinds of livestock and are fed predominantly on grass and locally grown fodder. Thus, cattle are the type of livestock most likely to prove good biomonitors of trace and contaminant metal concentrations in Galicia. The aim of this study was to investigate if the concentrations of metals and semimetals in the tissues of cattle were correlated with those in the soil in Galicia and to evaluate the feasibility of using cattle as biomonitors of environmental metal/semimetal levels in this region.

Material and Methods

Animal Sampling and Chemical Analysis

During June–November 1996, tissues were taken from a stratified random sample of calves from all of the slaughterhouses in Galicia. This was done to ensure that the sample collected was representative of the whole region. Information on the age, precise origin, and husbandry history of the animals was obtained from farm documentation. Only animals reared for their entire lives on the same farm and fed predominantly local forage were sampled. In total, tissues were taken from 438 (male and female) calves aged between 6 and 10 months. All were healthy at the time of slaughter.

Metal concentrations were determined in (1) the liver and kidney, because they are key organs that accumulate relatively high concentrations of certain elements; (2) muscle, because of its importance in foodstuffs; and (3) blood, because this allowed assessment of the use

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of a nondestructive sample to estimate soil metal/semimetal concentrations. Samples were collected at the time of slaughter and immediately transported to the laboratory where they were homogenized and frozen at −18°C until analysed. Approximately 1 g subsamples (2 ml of blood) were digested in concentrated nitric acid and 30% w/v hydrogen peroxide. Copper, zinc, and arsenic concentrations were determined by flame (copper/zinc) and hydride (arsenic) atomic absorption spectrophotometry. Lead and cadmium concentrations were also measured, but data are not presented because they could not be related to soil concentrations; there are no published data on the concentrations of these metals in soils across Galicia. All tissue copper and zinc concentrations were above the limit of detection. This was not the case for arsenic, and the limits of detection were 7.98, 7.20, and 6.50 μg/kg wet weight in liver, kidney, and muscle and 3.98 μg/L in blood. Analytical recoveries were determined from a certified reference material (pig kidney CRM 186, BCR Reference Materials) and from spiked samples. Certified mean concentrations for arsenic, copper, and zinc were 0.063, 31.9, and 128 mg/kg respectively and the corresponding measured (mean ± SD) concentrations in 34 samples were 0.056 ± 0.006, 31.6 ± 3.5, and 127.6 ± 10.5 mg/kg. Recoveries from spiked samples were similar to those from the CRM. Full details of the preparation of samples, analytical methods, and quality control data are given by López Alonso *et al.* (2000a).

Soil Data

Information on soil arsenic, copper, and zinc concentrations were obtained from the geochemical atlas of Galicia (Xunta de Galicia 1992). This contains data on metal concentrations in 27,288 soil samples and provides an average resolution of 1 sample per km^2 for Galicia (29,480 km^2); areas occupied by towns or cities, continental waters, and reservoirs are not covered. The soil samples (500 g each) were taken from the deep horizon in contact with the parent rock and were accompanied by a fragment of the subjacent rock. Each sample was dried and ground (maximum size 200 μm), and arsenic, copper, and zinc concentrations were determined by X-ray fluorescence. Galicia is a rural, relatively uncontaminated area, and atmospheric surface deposition of arsenic, copper, and zinc is low (Fernández and Carballeira 2001). Therefore, metal/semimetal concentrations in soil from the deep horizon are likely to be largely representative of residues in surface soils, apart from localized areas of anthropogenic contamination. For each element, soil atlas data were not given as precise values but were assigned to one of five (zinc) or six (arsenic, copper) class intervals.

Statistical Analysis

For each element, the class intervals for soil concentrations were considered for statistical purposes as independent groups. The provenance of each calf was located on the soil atlas and the tissue concentrations for that animal were assigned to the appropriate soil class-interval group. One-way analysis of variance (ANOVA) was used to determine whether there were significant differences in tissue arsenic, copper, and zinc concentrations between calves from areas with different soil levels. Copper and zinc (but not arsenic) residues in cattle vary with age (López Alonso *et al.* 2000a), but this did not confound analysis of the relations between soil and tissue residues. This was because there was no significant difference between the ages of animals from areas with different levels of copper ($F_{5,341} = 0.572$, $p > 0.05$) or zinc contamination ($F_{3,343} = 2.092$, $p > 0.05$).

Tissue concentration data for copper and zinc were log-transformed before they were analyzed to meet the underlying assumptions of the ANOVA. However, the variances in the tissue concentrations of arsenic differed markedly between groups, even after data were transformed. Therefore, untransformed arsenic data were analyzed by nonparametric Kruskal-Wallis test; the Kruskal-Wallis statistic ($H_{(df)}$) and the probability value are given to indicate the significance of the test. Because they were analysed nonparametrically, arsenic tissue concentration data are expressed as medians, interquartile, and total ranges (Table 1) whereas copper and zinc concentrations are given as log mean and SE values (Figures 1 and 2).

Results

The concentrations of arsenic in Galician soils ranged from less than 1 to more than 20 mg/kg. There was significant variation in liver and kidney arsenic residues between calves from areas with different levels of soil arsenic (liver: $H_{(5)} = 221$, $p < 0.001$; kidney: $H_{(5)} = 177$ $p < 0.001$; Table 1), but this was solely because animals from areas with soil arsenic concentrations above 20 mg/kg had elevated tissue residues. For calves from all the other areas, even the upper quartile value for tissue arsenic concentration was at or below the detection limit. Arsenic concentrations in muscle were generally low, and fewer than 25% of animals from each area had residues above the detection limit. However, the differences in muscle residues between animals from different areas were statistically significant ($H_{(5)} = 21.5$, $p < 0.001$) and the upper range in muscle arsenic concentrations increased progressively with soil arsenic concentrations (Table 1). There was no significant difference ($H_{(5)} = 9.54$, $p > 0.05$) in blood arsenic concentration between animals from different areas.

Copper concentrations in Galician soils varied from below 5 mg/kg to over 150 mg/kg (Figure 1). Copper concentrations in the liver varied significantly between animals from different areas ($F_{(5,431)} = 14.6$, $p < 0.001$), and mean concentrations increased progressively with the copper content of the soil (Figure 1). Copper concentrations in the kidney and muscle did not vary significantly between animals from different areas ($F \leq 0.43$, $p > 0.05$ in both cases). Mean blood copper concentrations tended to be higher in animals from areas where soil copper levels exceeded 100 mg/kg (Figure 1), but overall there was no significant variation in blood copper levels between animals from different areas ($F_{(5,370)} = 0.42$, $p > 0.05$).

Soil zinc concentrations ranged from less than 25 mg/kg to greater than 400 mg/kg in Galicia. There are few areas in Galicia where soil zinc concentrations are less than 25 mg/kg, and none of the sampled animals were from such areas. Liver zinc residues varied significantly between animals from different areas ($F_{(3,433)} = 6.64$, $p < 0.001$), higher residues being found in animals with the greatest levels of zinc in the soil (Figure 2). Zinc concentrations in kidney, muscle and blood did not vary significantly between animals from different areas ($F \leq 1.61$, $p > 0.05$ in all cases).

Discussion

Previous studies have shown that grazing animals fed locally produced fodder reflect the environmental concentrations of toxic elements in contaminated areas (Ronneau *et al.* 1983; Ronneau and Cara 1984; Morcombe *et al.* 1994; Petersson Grawé *et al.* 1997). In Galicia, calves mainly eat local forage, so it would be expected that the main route of exposure to

Table 1. Arsenic concentrations in the liver, kidney, muscle (fflg/kg wet weight) and blood (fflg/L) in Galician cattle form areas with differing levels of arsenic in the soil

	Soil Arsenic Concentration (mg/kg)					
	– 1	1–2	2–5	5–10	10–20	ff 20
Liver						
No. calves	18	31	105	83	13	187
Median	ND	ND	ND	ND	ND	71.5
Interquartile range	ND–ND	ND–ND	ND–ND	ND–ND	ND–ND	ND–159
Range	ND–72.8	ND–ND	ND–76.3	ND–65.5	ND–ND	ND–401
Kidney						
No. calves	18	28	102	84	12	183
Median	ND	ND	ND	ND	ND	76.8
Interquartile range	ND–ND	ND–ND	ND–ND	ND–ND	ND–ND	ND–154
Range	ND–147	ND–10.4	ND–221	ND–274	ND–9.58	ND–537
Muscle						
No. calves	18	31	105	84	13	187
Median	ND	ND	ND	ND	ND	ND
Interquartile range	ND–ND	ND–ND	ND–ND	ND–ND	ND–ND	ND–ND
Range	ND–ND	ND–9.35	ND–13.3	ND–14.8	ND–19.0	ND–24.5
Blood						
No. calves	14	27	87	73	10	166
Median	ND	ND	ND	ND	ND	ND
Interquartile range	ND–5.57	ND–ND	ND–ND	ND–ND	ND–ND	ND–6.51
Range	ND–44.0	ND–30.5	ND–31.4	ND–54.7	ND–33.8	ND–49.8

ND: nondetectable.

metals would be from direct ingestion of soil and from food-chain transfer of elements through forage. Our results demonstrate that associations between residues in soil and animals can be detected in calves over large, relatively uncontaminated areas (the whole region of Galicia) for the toxic semimetal arsenic and for the essential trace metals copper and zinc. The highest tissue concentrations of each element were usually accumulated in the liver, and variation in hepatic concentrations reflected the differences in soil element concentrations between the areas in which the calves were reared. This was not generally true for kidney or muscle concentrations, however. Several studies have demonstrated that concentrations of some metals in blood are good indicators of recent exposure of livestock (Takla *et al.* 1989; Telisman *et al.* 1990; Humphreys 1991; Kalavska 1992; Palheta and Taylor 1995; Gummow 1996; Cerna *et al.* 1997), but in our study there was no association between soil arsenic, copper, or zinc and blood concentrations in calves. This was perhaps because in Galicia, soil concentrations of these elements were not generally elevated above the normal background range, and blood may not be a good nondestructive biomonitor of variation in background concentrations.

A significant association between environmental arsenic concentrations and tissue residues in cows has been found previously in animals close to a smelter (Jenkins 1980). However, arsenic concentrations in soils throughout Galicia are in the background range (– 40 mg/kg dry weight arsenic; Eisler 1994). Appreciable arsenic concentrations were only detected in the livers of calves from areas with soil arsenic concentrations above 20 mg/kg; residues in calves from all other areas were mostly low or undetectable. This suggests that cattle may only prove useful biomonitors of soil arsenic concentrations greater than 10–20 mg/kg, and variation in their tissue residues will not reflect differences between areas with lower background concentrations.

Copper, like other essential trace metals, is normally under tight homeostatic control in mammals (Piscator 1979; Davis and Mertz 1987), so it might not normally be expected that tissue residues would reflect soil concentrations. However, in contrast to other mammals, ruminants have a superior capacity to bind copper in the liver and have relatively poor copper excretion; this results in a level of copper storage in the liver 10 times that of other species (Davis and Mertz 1987). When dietary levels of copper are high, liver copper concentrations in cattle increase proportionally with the amount of metal ingested (Gummow 1996). This occurs because metallothioneins and other ligands sequester the copper, although they can become saturated and additional copper then complexes with and damages microsomes and other proteins, causing rapid hepatic cell death, a subsequent release of copper into the circulation, and a potentially fatal hemolytic crisis (Brewer 1987; Davis and Mertz 1987; Radostits *et al.* 2000). The characteristic of high copper storage capacity in ruminants means that they are likely to be good biomonitors of environmental copper levels and can be used to identify areas of copper contamination. This was demonstrated by our study in which the variation in copper concentration in the livers of calves matched the wide-scale natural variation in the soil that results from the presence of underlying, ultrabasic serpentines in some areas of Galicia. Furthermore, the detection of high copper concentrations in the livers of calves from one region of Galicia where soil copper levels were known to be within the normal range (5–25 mg/kg) has allowed us to identify a zone of copper pollution caused by the fertilizing of grazing pasture with copper-enriched pig slurry (López Alonso *et al.* 2000b).

Copper status and metabolism in ruminants is strongly af-

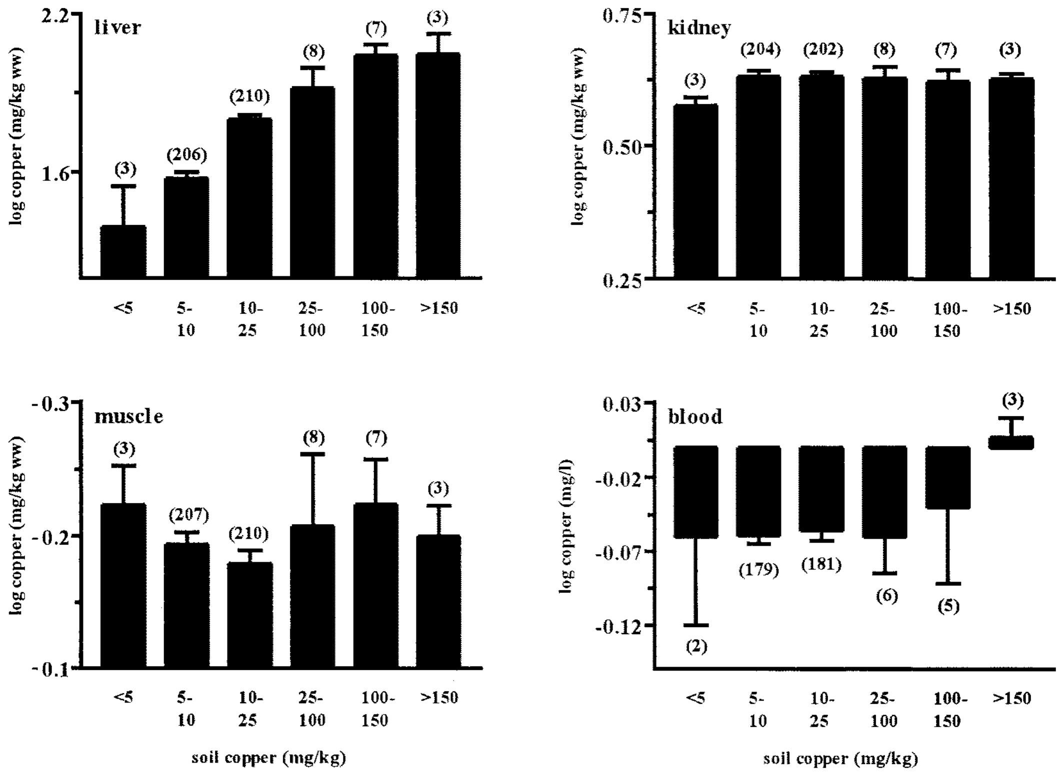

Fig. 1. Mean ┘ SE log copper concentrations in the liver, kidney, muscle (each expressed as mg/kg ww) and blood (mg/L) in Galician cattle from areas with differing soil copper concentrations. Number of samples in each group is given in parentheses. Note values for blood copper are negative because data are logged

fected by other essential elements, particularly molybdenum (Davis and Mertz 1987; Wikse *et al.* 1992; Radostits *et al.* 2000) as high and low molybdenum intakes leading to copper deficiency and toxicity respectively, even when dietary copper levels are normal (Gooneratne *et al.* 1989). We do not have direct measures of the copper and molybdenum intake of the calves in our study, but soil molybdenum levels in Galicia are in the normal background range (0.1–1 mg/kg; Xunta de Galicia 1992), are relatively uniform throughout the region and did not vary significantly between areas with different soil copper levels ($H_{(5)}$ | 3.97, p ff 0.05). Thus the observed variation in hepatic copper concentrations between calves from areas with different soil copper levels was not attributable to potential differences in molybdenum intake. However, the use of calves as biomonitors for copper may be limited to regions (such as Galicia) in which there is little variation in soil concentrations of molybdenum and other trace metals that strongly affect copper metabolism.

Zinc is another essential trace metal and body tissue concentrations are regulated by homeostatic mechanisms in all mammals, including ruminants (Elinder and Piscator 1979; NRC 1980). Thus, mammals are not usually considered suitable as biomonitors of environmental variation in zinc concentrations. Koh and Judson (1986) did not find significant differences in liver zinc concentrations between sheep exposed to different concentrations of the metal in the diet, suggesting that zinc concentrations in the liver are not reliable indicators of intake levels in ruminants. In the present study, there was a positive association between the concentrations of zinc in the soil and the residue levels in the livers of calves, mean liver zinc concentrations being greater in animals from areas with higher levels of zinc in the soil. However, copper and zinc levels were positively correlated with each other both in soil (Spearman's rank correlation coefficient, Rs | 0.394, p − 0.001, n | 437) and in calf liver (Pearson's product moment correlation coefficient, $r_{(491)}$ | 0.107, p − 0.05). Therefore, it is possible that the apparent relation between soil and liver zinc residues could be influenced by interactions between the two metals in the soil and/or in the liver and there may not necessarily be any causal association between zinc levels in the soil and in calves.

Conclusions

It would appear that calves may potentially be good biomonitors of soil concentrations of arsenic, copper, and zinc in Galicia, as liver residues were positively associated with concentrations in soils. There may be similar associations for other metals, particularly nonessential metals, such as cadmium and lead, but we could not investigate these because of the lack of data on these elements in Galician soils. In general, calves in Galicia fulfill the various selection criteria required of biomonitors (Holden 1973; Landres *et al.* 1988; O'Brien *et al.* 1993; Esselink *et al.* 1995) and it is possible that they would also prove good biomonitors in other regions and countries that have similar cattle husbandry practices and soil types.

The use of cattle as biomonitors has specific advantages over

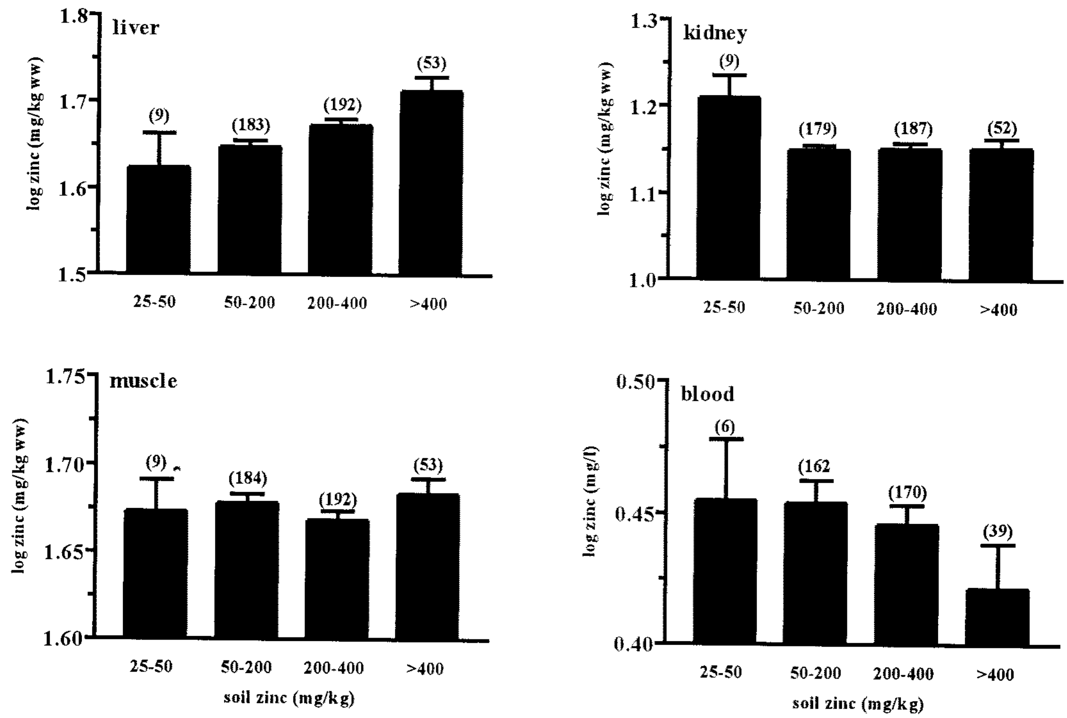

Fig. 2. Mean ± SE log zinc concentrations in the liver, kidney, muscle (each expressed as mg/kg ww) and blood (mg/L) in Galician cattle from areas with differing soil zinc concentrations. Number of samples in each group is given in parentheses

direct measurement of soil samples. Variation in liver residues between calves gives an indication of the variation in bioavailable (rather than total) metal levels in the environment and, arguably, are more useful measures. Liver residues are also an integrated measure of element concentration over the foraging area of the calf and so may be less prone than soil cores to large intersample variations caused by small-scale heterogeneity in the distribution of elements within the soil. Additionally, because farmers are required to keep good animal records and to take calves to slaughterhouses for butchering, well-documented samples are routinely collected at a relatively small number of sites, making sampling of environmental element concentration across wide spatial scales easy and cheap compared with field sampling of soil cores.

The reporting of Galician soil data on a class-interval basis, the restricted number of contaminants for which there are data, and the relatively uncontaminated nature of Galician soils has limited the extent to which we have been able to analyze the relationship between soil metal levels and liver residues in calves. If calves are to be valuable as biomonitors of soil contamination, this relationship requires better definition using regression models that will produce confidence limits on predicted soil metal concentrations. Development of such simple models requires actual concentration data for each soil sample (rather than a value grouped into a class interval); these data were not available to us for the present study. It is also necessary to examine the robustness of these relationships in terms of how they may be affected by other elements and environmental factors. However, this study has demonstrated that the approach of using calves as biomonitors of soil concentrations of metals and semimetals is tractable and may offer considerable advantages over direct soil sampling, particularly when data for large areas are required.

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